SUPPORTING INFORMATION

Organelle-selective Energy Transfer: An Indicator Of Intracellular Environment

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General Experimental Methods

All reactions were carried out under an atmosphere of dry nitrogen. Glassware were oven-dried prior to use. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification. All solvents were dried prior to use with appropriate drying agents. Dry distilled DMF was obtained from Acros and used as such. Flash column chromatography was performed using silica gel 60 (230-400 mesh). Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV. Fluorescence spectra were obtained on a Varian Cary Eclipse fluorescence spectrophotometer at room temperature. Absorbance spectra were obtained on a Varian 100 Bio UV-Vis spectrophotometer at room temperature.

^{1}H and ^{13}C spectra were recorded on a Varian 300 (300 MHz ^{1}H; 75 MHz ^{13}C) or Varian 500 (500 MHz ^{1}H; 125 MHz ^{13}C) spectrometer at room temperature. Chemical shifts were reported in ppm relative to the residual DMSO-d_6 (δ 2.54 ppm ^{1}H; δ 40.45 ppm ^{13}C). ^{19}F NMR were acquired on a Varian 300 (300 MHz ^{1}H; 282 MHz ^{19}F) spectrometer. $CFCl_3$ was used as an external reference for the ^{19}F NMR spectra. Coupling constants (*J*) were reported in Hertz.

Photophysical Properties and Determination of Quantum Yields

Steady-state fluorescence spectroscopic studies were performed on a Cary Eclipse fluorometer. The slit width was 5 nm for both excitation and emission. The relative quantum yields of the samples were obtained by comparing the area under the corrected emission spectrum of the test sample with that of a standard dye.[1] The quantum efficiencies of fluorescence were obtained from three measurements with the following equation:

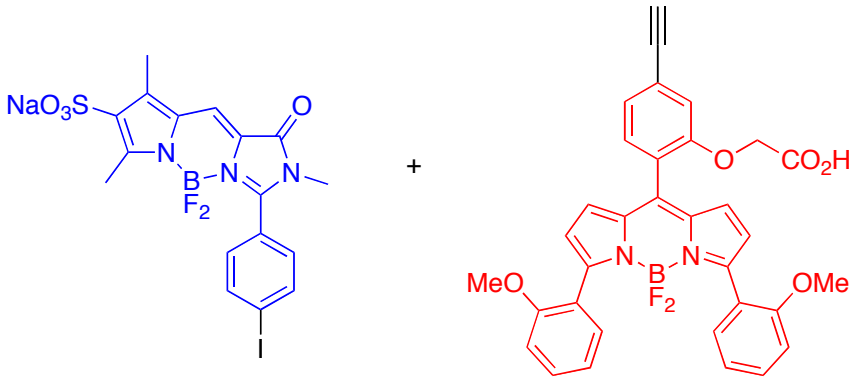
$$\Phi_x = \Phi_{st} (I_x/I_{st}) (A_{st}/A_x) (\eta_x^2/\eta_{st}^2)$$

Where Φ_{st} is the reported quantum yield of the standard, **I** is the area under the emission spectra, **A** is the absorbance at the excitation wavelength and η is the refractive index of the solvent used, measured on a pocket refractometer from ATAGO. **X** subscript denotes unknown, and **st** denotes standard.[2]

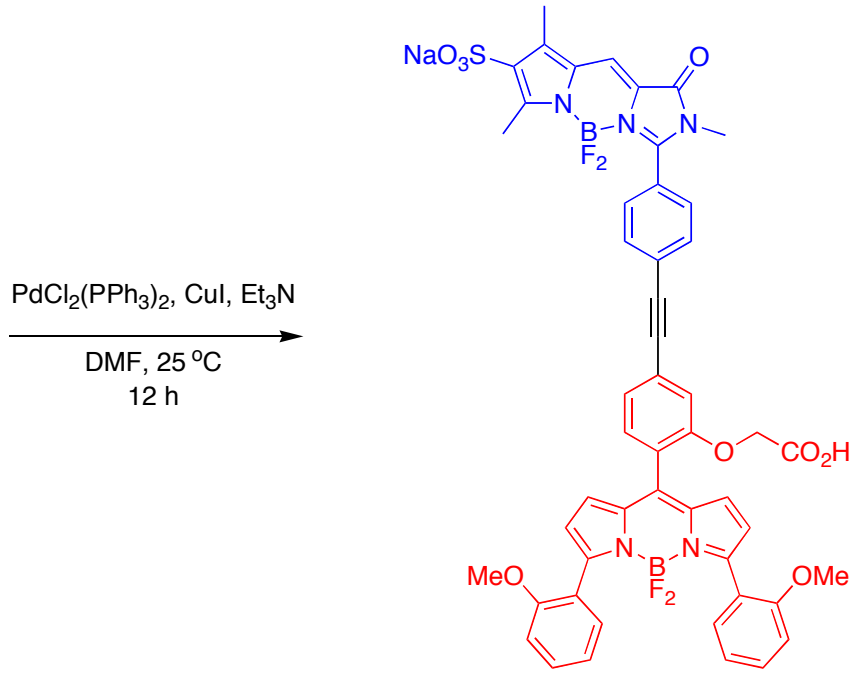
Syntheses of Energy Transfer Cassettes

Syntheses of the cassettes **1** and **2** were accomplished via Sonogashira cross couplings[3] of the relevant fragments (Scheme S1).

a

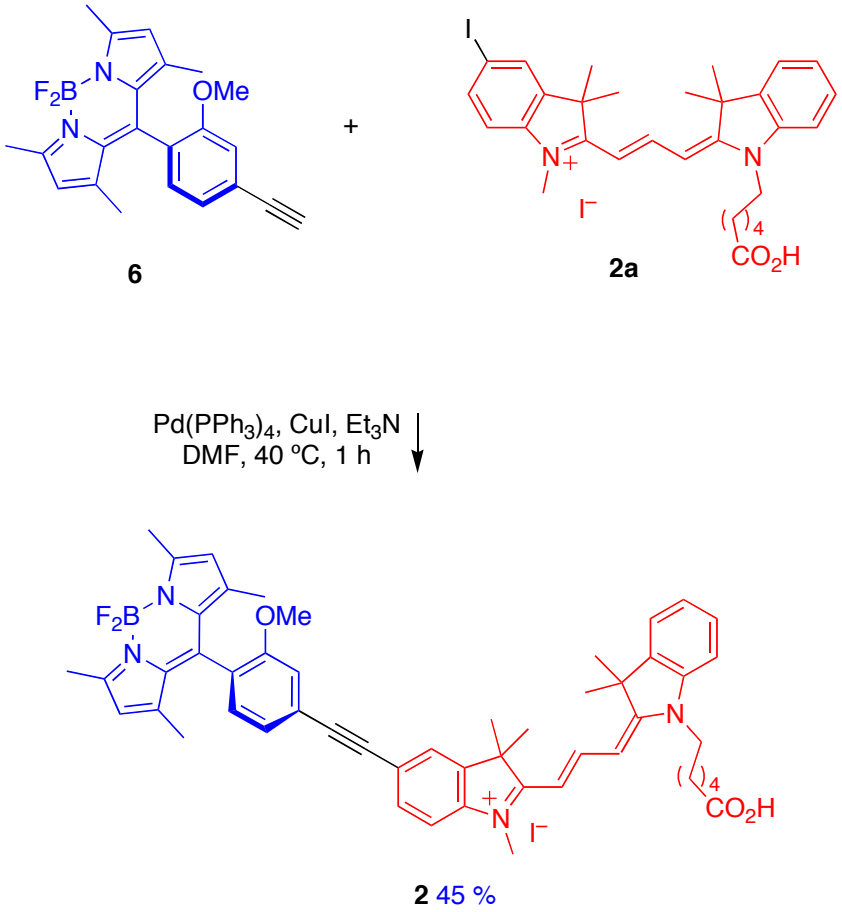


5 **1a**



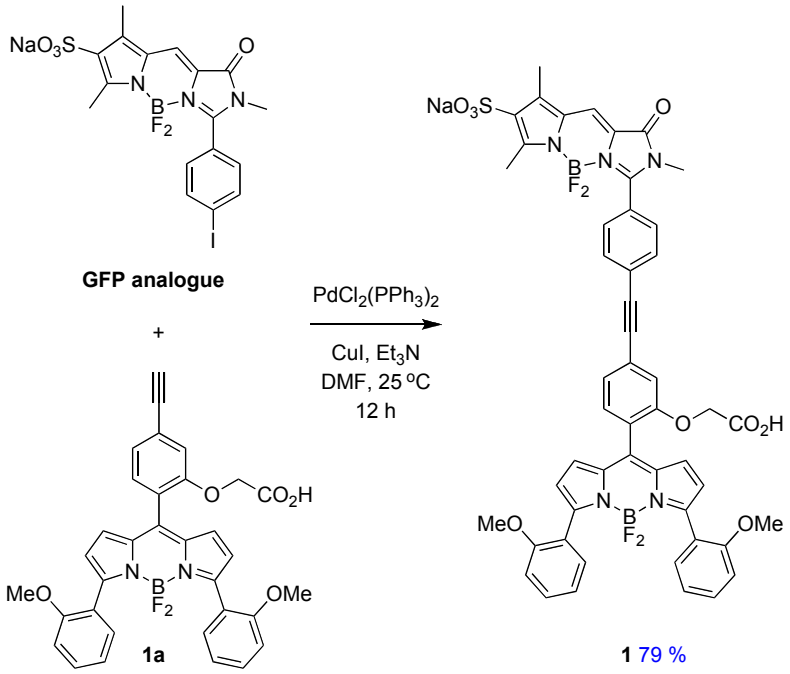
1 79 %

b



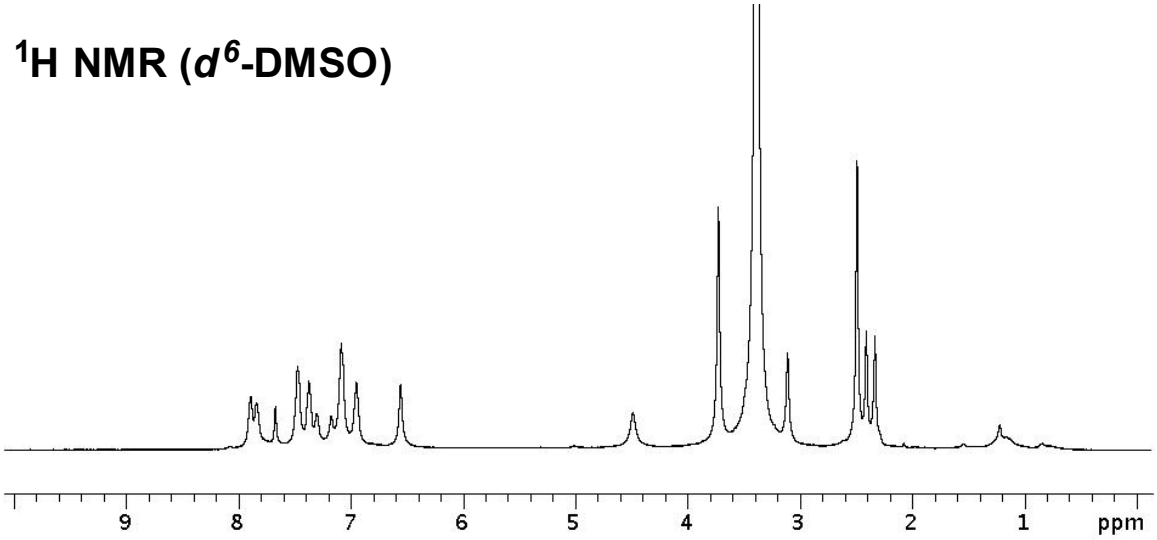
Scheme S1. Routes to target compounds: **a** synthesis of cassette **1**; **b** synthesis of cassette **2**.

Synthesis of Cassette 1

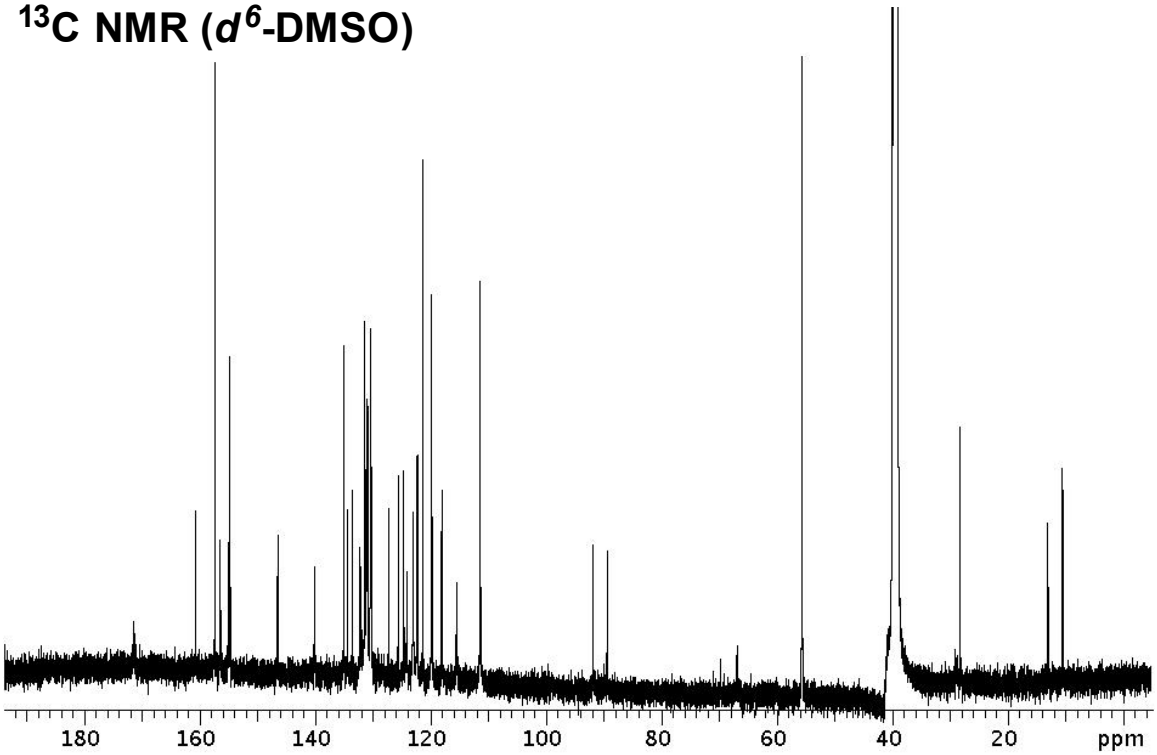


A solution of donor (**GFP analogue**)[3] (14 mg, 0.025 mmol), BODIPY acceptor **1a** (20 mg, 0.035 mmol), $PdCl_2(PPh_3)_2$ (2 mg, 0.003 mmol) and CuI (0.6 mg, 0.003 mmol) in 3 mL DMF was degassed and filled with N_2 gas then Et_3N (35 μL, 0.25 mmol) was added. The reaction mixture was stirred at 25 °C for 24 h then concentrated under reduced pressure. The residue was purified by flash chromatography (20 % MeOH/CH_2Cl_2) to afford the product **1** (20 mg, 79 %) as a red solid. ^{1}H NMR (500 MHz, *d*^{6}-DMSO) δ 7.89-7.84 (m, 3H), 7.67 (s, 1H), 7.47 (br, 3H), 7.37-7.30 (m, 4H), 7.17 (s, 1H), 7.08-7.07 (m, 4H), 6.95 (br, 2H), 6.56 (br, 2H), 4.48 (s, 2H), 3.73 (s, 6H), 3.11 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (500 MHz, *d*^{6}-DMSO) δ 171.3, 160.7, 157.3, 156.4, 155.0, 154.7, 146.4, 140.1, 140.0, 135.0, 134.4, 133.5, 132.2, 131.4, 131.1, 130.9, 130.4, 130.2, 127.2, 125.6, 124.6, 124.1, 123.0, 122.3, 121.3, 119.8, 118.1, 117.9, 115.5, 111.4, 91.8, 89.4, 66.9, 55.6, 28.2, 13.0, 10.5; MS (ESI) m/z calcd for $(M\text{-}Na)^-$ $C_{50}H_{38}B_2F_4N_5O_9S$ 982.25; found 982.25. m/z calcd for $(M\text{-}Na\text{-}H)^{2-}$ $C_{50}H_{37}B_2F_4N_5O_9S$ 490.62; found 490.62.

^{1}H NMR (d^{6}-DMSO)

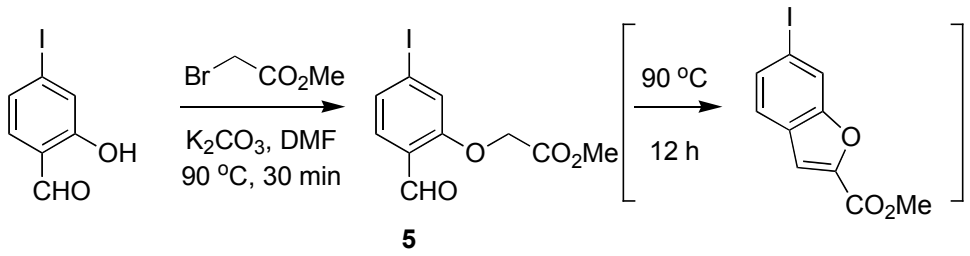


^{13}C NMR (d^{6}-DMSO)



Syntheses of **1a**

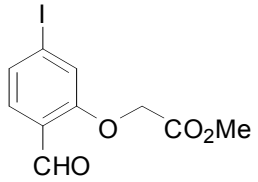
Benzaldehyde derivative



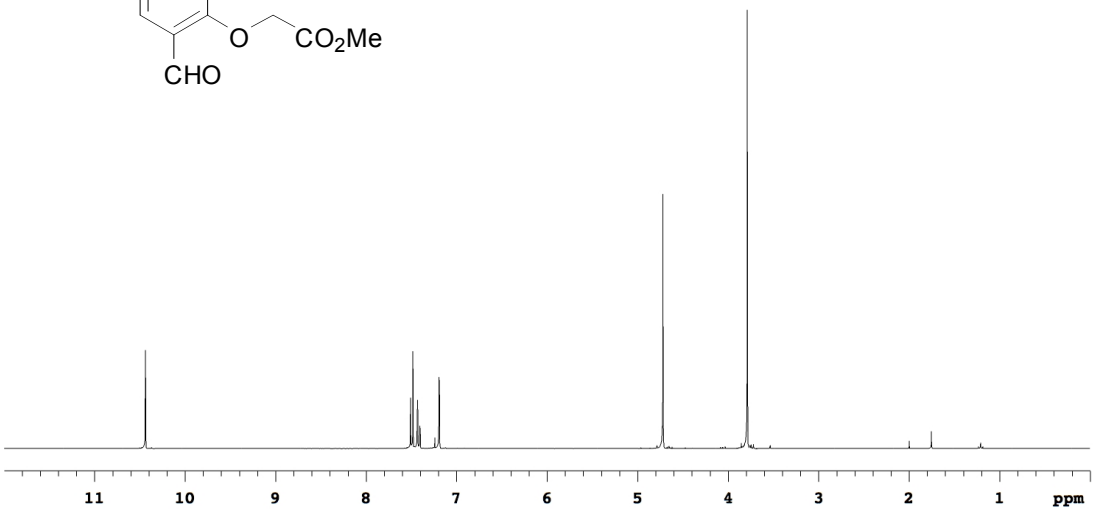
5

2-Hydroxy-4-iodobenzaldehyde (1.24 g, 5.0 mmol) and K_2CO_3 (1.38 g, 10.0 mmol) were dissolved in 25 mL DMF and stirred for 10 min then methyl bromoacetate (0.7 mL, 7.5 mmol) was added. The mixture was stirred at 90 °C for 30 min then cooled to room temperature and diluted with EtOAc. The mixture was washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (10 to 20 % EtOAc/Hexanes) to afford the product **5** as white solid (1.41 g, 88 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.44 (s, 1H), 7.49 (d, 1H, J = 8.1 Hz), 7.43-7.40 (m, 1H), 7.19 (d, 1H, J = 1.4 Hz), 4.72 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.8, 168.0, 159.6, 131.4, 129.4, 124.8, 122.1, 102.8, 65.5, 52.5.

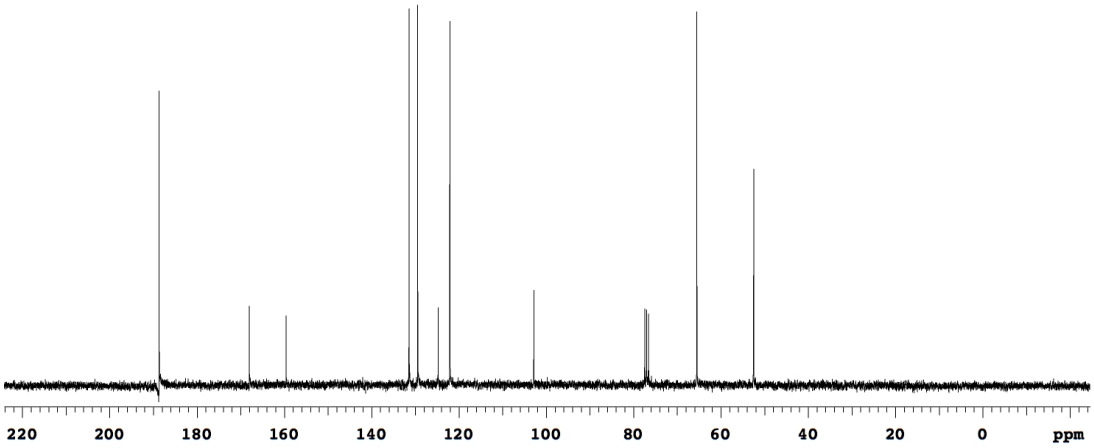
If the mixture was stirred at 90 °C for 12 h, the reaction gave a different product which was found to be a benzofuran derivative in 62 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.57 (dd, 1H, J = 8.2, 1.4 Hz), 7.43 (d, 1H, J = 1.4 Hz), 7.38 (d, 1H, J = 8.2 Hz), 3.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 155.8, 145.5, 133.0, 126.4, 124.0, 121.6, 113.6, 91.9, 52.5.



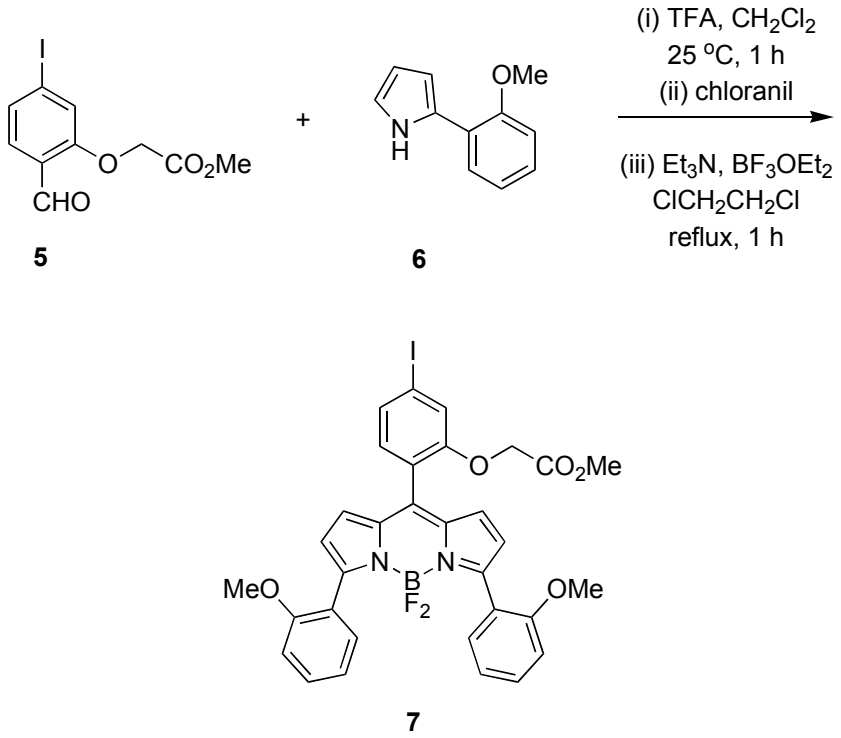
^{1}H NMR ($CDCl_3$)



^{13}C NMR ($CDCl_3$)

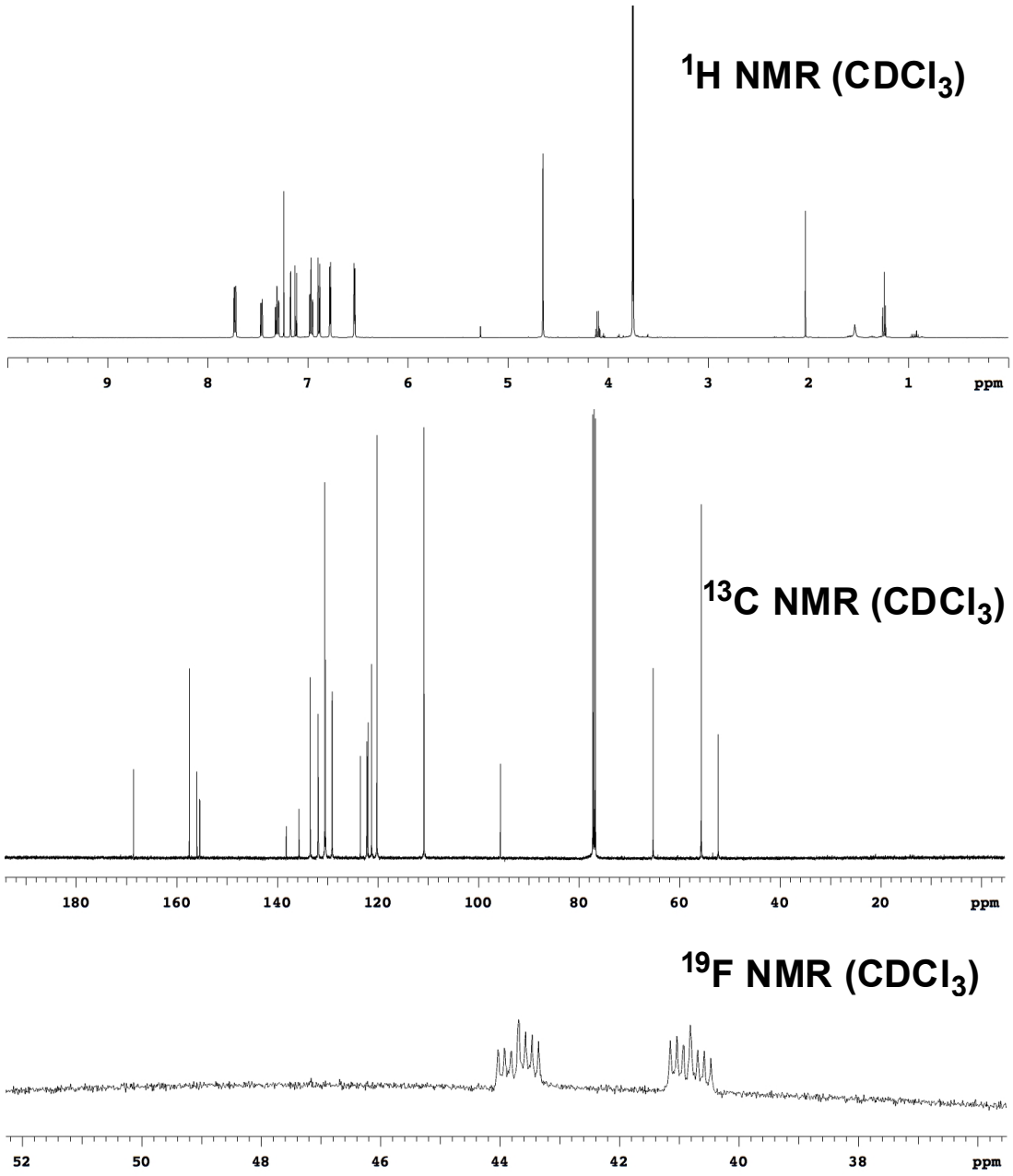


Synthesis of BODIPY 7

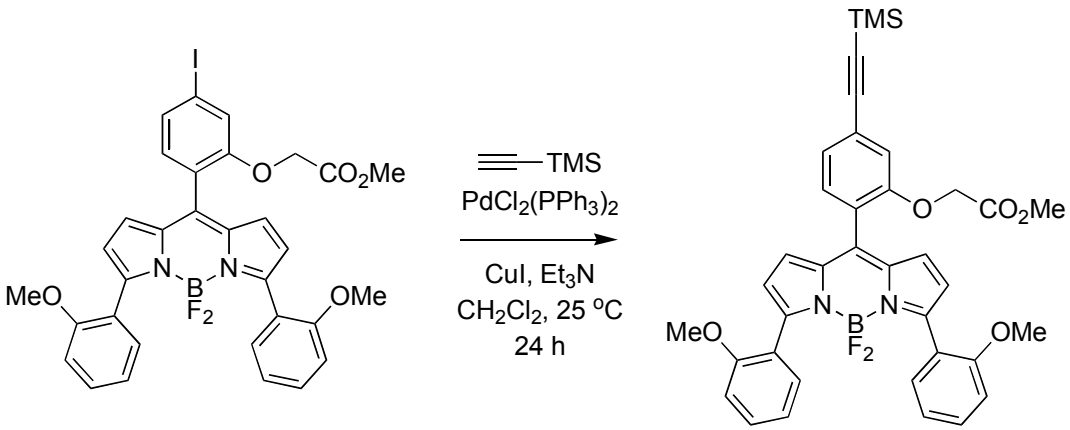


Compound **5** (320 mg, 1 mmol) and pyrrole **6** (346 mg, 2 mmol) were dissolved in 10 mL CH_2Cl_2 and one drop of TFA was added. The solution was stirred at room temperature until TLC showed the complete consumption of the aldehyde (around 1 h). Chloranil (246 mg, 1 mmol) was added and stirring was continued for 30 min. The mixture was passed through a short pad of basic alumina eluting with CH_2Cl_2. The solvents were removed under reduced pressure. The residue was dissolved in 20 mL 1,2-dichloroethane and Et_3N (0.42 mL, 3 mmol) was added followed by BF_3OEt_2 (0.63 mL, 5 mmol) after 10 min. The solution was refluxed for 1 h then cooled to room temperature. The reaction mixture was washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (20 to 40 % EtOAc/Hexanes) to afford the product (480 mg, 69 %) as red solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74-7.72 (m, 2H), 7.46 (dd, 1H, *J* = 7.9, 1.3 Hz), 7.32-7.29 (m, 2H), 7.17 (d, 1H, *J* = 1.3 Hz), 7.12 (d, 1H, *J* = 7.9 Hz), 6.98-6.95 (m, 2H), 6.89 (d, 2H, *J* = 8.3 Hz), 6.78 (d, 2H, *J* = 4.3 Hz), 6.53 (d, 2H, *J* = 4.3 Hz), 4.65 (s, 2H), 3.76 (s, 3H), 3.75 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.6, 157.5, 156.0, 155.4, 138.2, 135.7, 133.4, 131.9 (t, *J* = 5.3 Hz), 130.6, 130.4, 129.1, 123.5, 122.2, 121.9, 121.3, 120.2, 110.8, 95.7, 65.3,

55.7, 52.3; ^{19}F NMR (282 MHz, $CDCl_3$) δ 43.69 (dq, 1F, J = 97.2, 30.8 Hz), 40.81 (dq, 1F, J = 97.2, 30.8 Hz); MS (ESI) m/z calcd for $(M+H)^+$ $C_{32}H_{27}BF_2IN_2O_5$ 695.10; found 695.11.

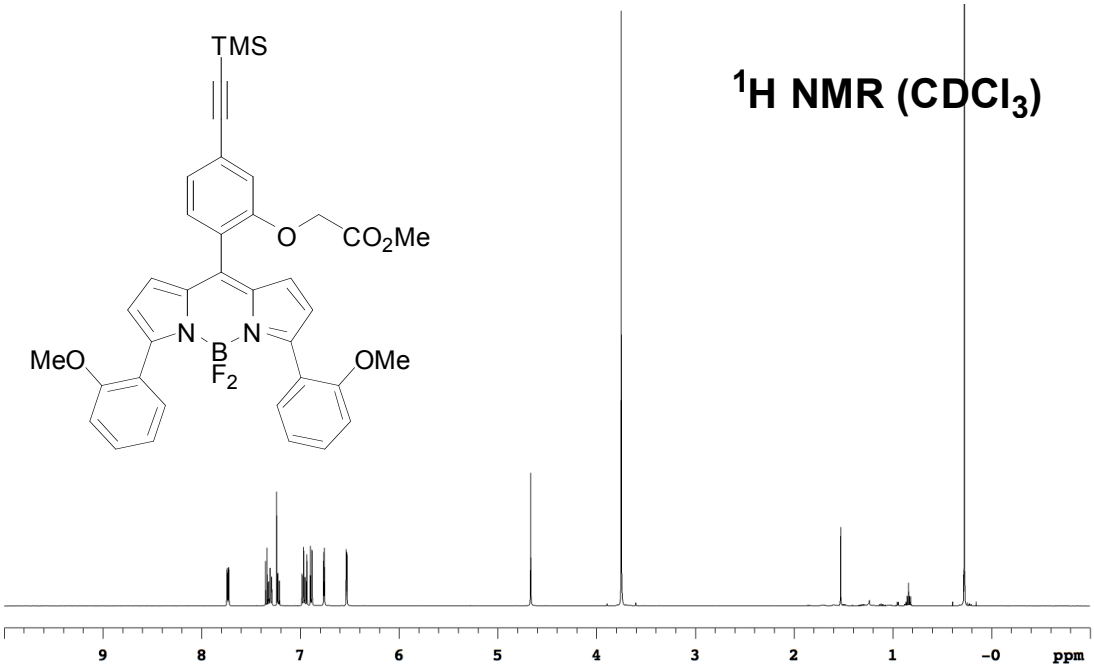


*Synthesis of BODIPY **8***



7 **8**

BODIPY **7** (208 mg, 0.3 mmol), trimethylsilylacetylene (0.4 mL, 3.0 mmol), $PdCl_2(PPh_3)_2$ (11 mg, 0.02 mmol), CuI (3 mg, 0.02 mmol) were dissolved in 20 mL CH_2Cl_2 then Et_3N (0.4 mL, 3.0 mmol) was added. After stirring at room temperature for 24 h, the reaction mixture was evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (20 to 30 % EtOAc/Hexanes) to afford the product **8** (184 mg, 92 %) as red solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74-7.72 (m, 2H), 7.34 (d, 1H, *J* = 7.8 Hz), 7.32-7.29 (m, 2H), 7.22 (dd, 1H, *J* = 7.8, 1.3 Hz), 6.98-6.95 (m, 2H), 6.93 (d, 1H, *J* = 1.3 Hz), 6.89 (d, 2H, *J* = 8.3 Hz), 6.76 (d, 2H, *J* = 4.3 Hz), 6.53 (d, 2H, *J* = 4.3 Hz), 4.67 (s, 2H), 3.75 (s, 9H), 0.28 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.9, 157.5, 155.3 (2 peaks: 155.33, 155.26), 138.7, 135.7, 132.2, 131.9 (t, *J* = 5.4 Hz), 130.5, 129.1, 125.6, 124.9, 124.2, 122.1, 122.0, 120.2, 114.9, 110.8, 104.0, 96.2, 65.2, 55.7, 52.3, -0.1; MS (MALDI) m/z calcd for M^+ $C_{37}H_{35}BF_2N_2O_5Si$ 664.24; found 664.31.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CO2Me
N
B
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F2
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OMe
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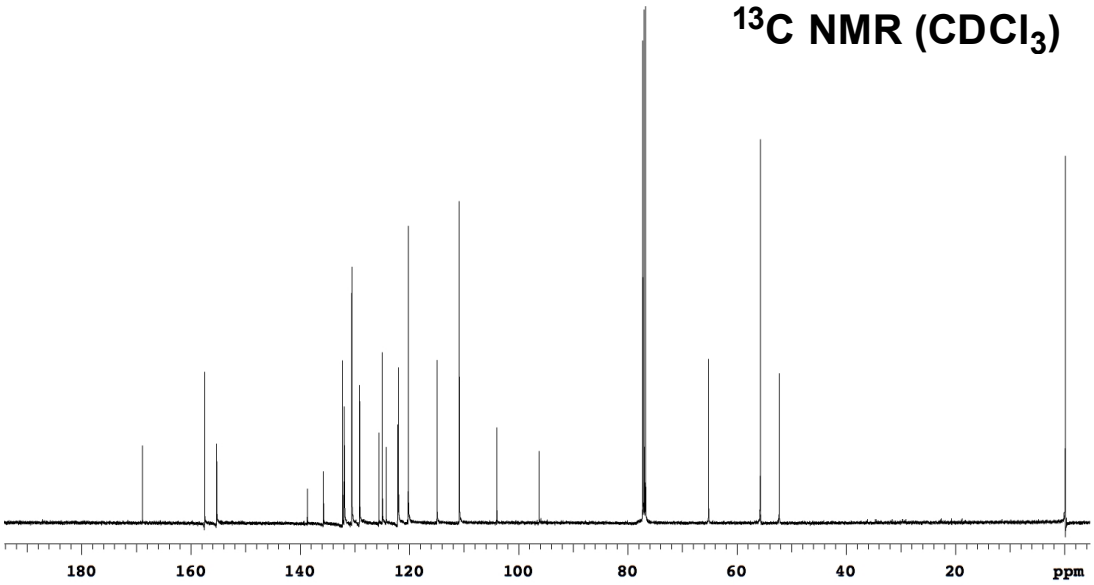
ppm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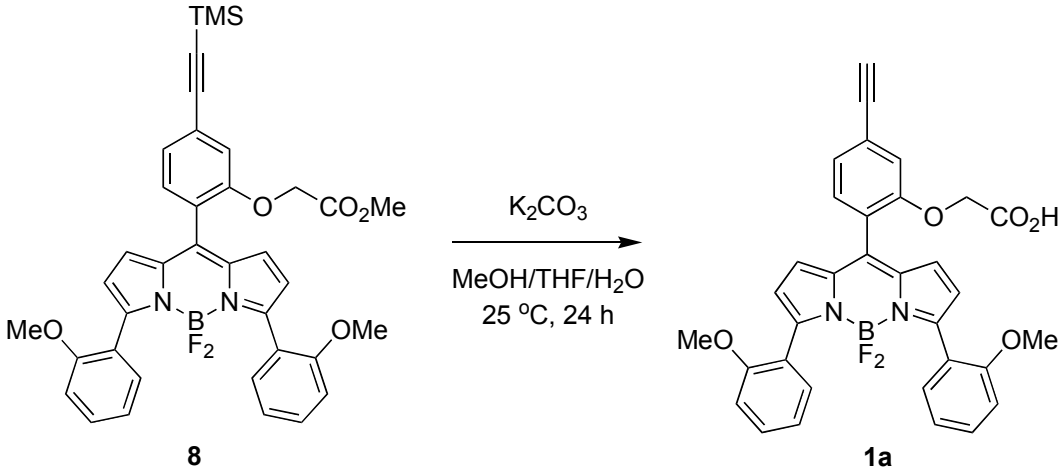
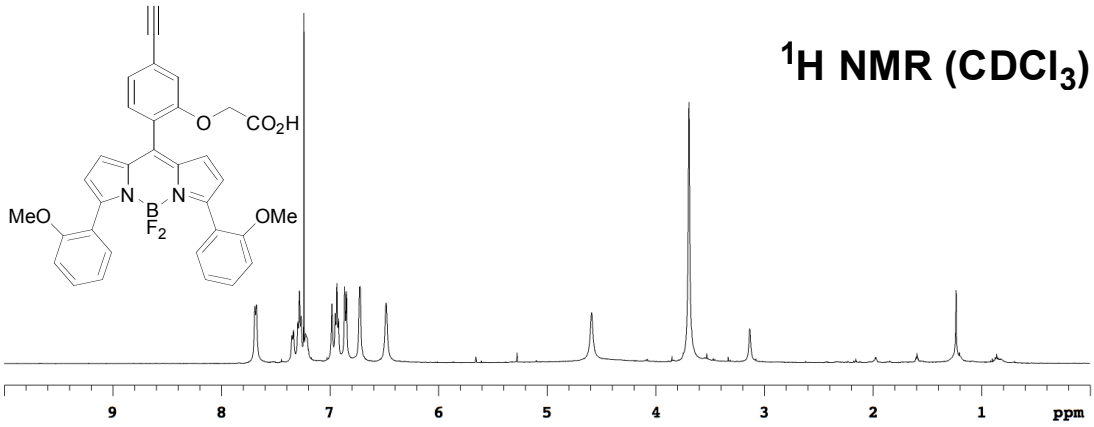
160
140
120
100
80
60
40
20
ppm

*Synthesis of BODIPY **1a***



BODIPY **8** (66 mg, 0.1 mmol) and K_2CO_3 (69 mg, 0.5 mmol) were dissolved in 5 mL MeOH + 5 mL THF + 1 mL H_2O. After stirring at room temperature for 24 h, the reaction mixture was diluted with H_2O, acidified with 2 M HCl and extracted with CH_2Cl_2. The organic layer was washed with H_2O, brine and dried over Na_2SO_4 then evaporated to dryness. The residue was purified by flash chromatography (2 to 5 % MeOH/CH_2Cl_2) to afford the product **1a** (49 mg, 85 %) as red solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69-7.68 (m, 2H), 7.35-7.34 (m, 1H), 7.30-7.26 (m, 2H), 7.23-7.21 (m, 1H), 6.98 (s, 1H), 6.95-6.92 (m, 2H), 6.86-6.85 (m, 2H), 6.72 (br, 2H), 6.48 (br, 2H), 4.59 (s, 2H), 3.69 (s, 6H), 3.13 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 157.4, 155.5, 155.1, 138.3, 135.6, 132.3, 131.8, 130.6, 129.1, 125.1, 124.7, 124.4, 122.3, 121.8, 120.2, 115.4, 110.9, 82.6, 79.1, 65.1, 55.7; ^{19}F NMR (282 MHz, $CDCl_3$) δ 43.73 (dq, 1F, J = 97.6, 30.7 Hz), 40.83 (dq, 1F, J = 97.6, 30.7 Hz); MS (ESI) m/z calcd for $(M-H)^-$ $C_{33}H_{24}BF_2N_2O_5$ 577.17; found 577.21.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MeO
OMe
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7
6
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2
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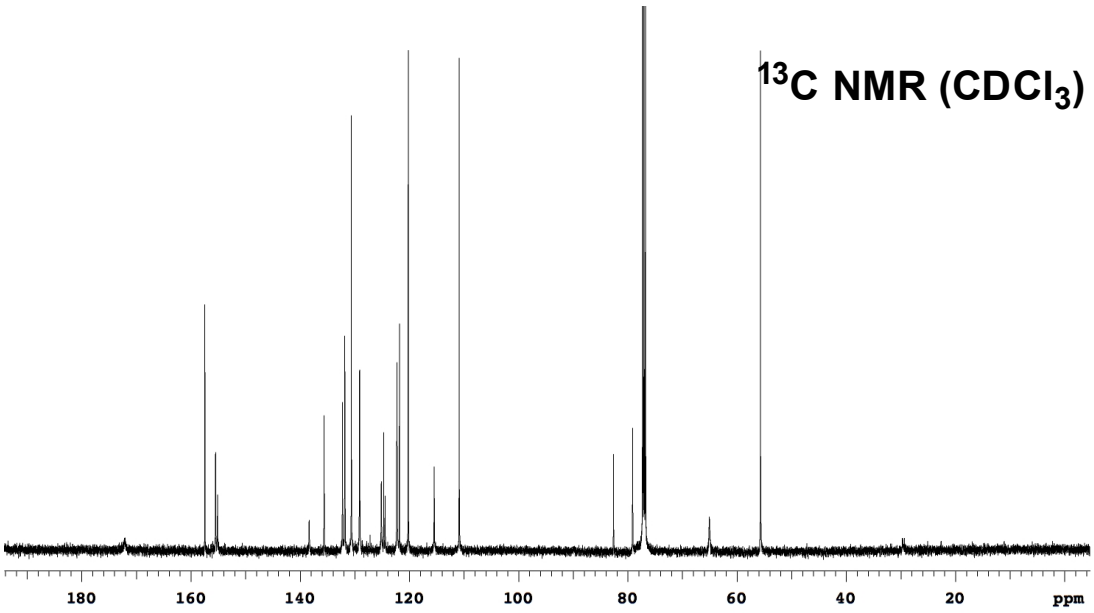
ppm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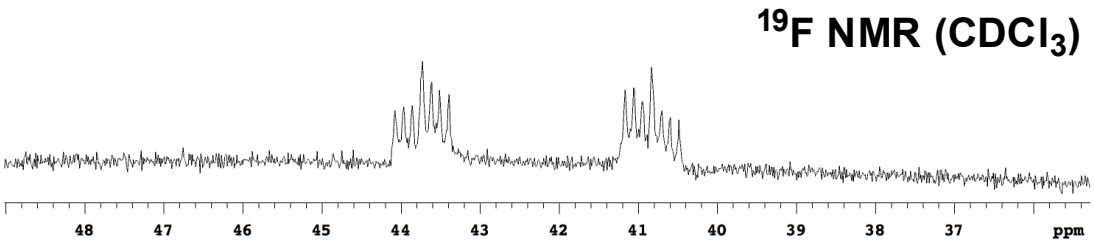
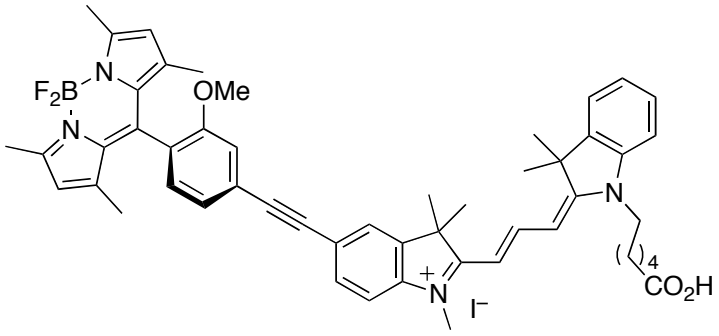
140
120
100
80
60
40
20
ppm

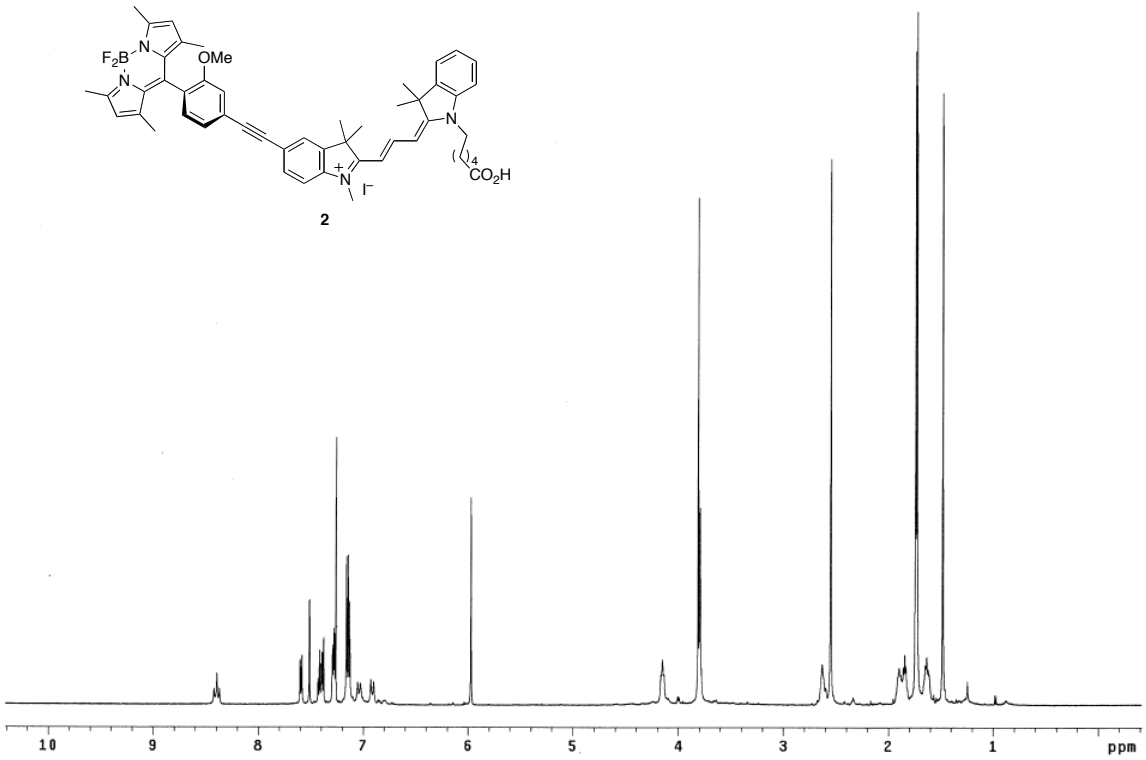

^{19}F NMR ($CDCl_3$)
48
47
46
45
44
43
42
41
40
39
38
37
ppm

Synthesis of Cassette **2** *(detailed synthesis of the donor **D** and acceptor **2a** will be reported elsewhere)*



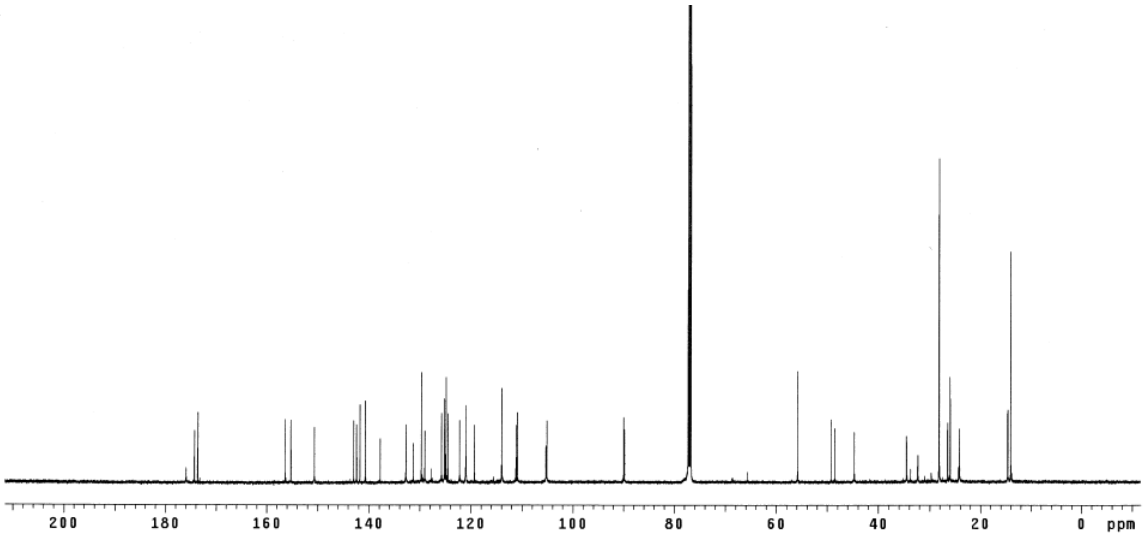
2

A solution of 5-iodo-Cy3 **2a** (70 mg, 0.099 mmol), 4-ethynyl BODIPY **D** (63 mg, 0.17 mmol), $Pd(PPh_3)_4$ (23 mg, 0.020 mmol), CuI (3.8 mg, 0.020 mmol) in DMF (4.0 mL) was freeze-pump-thawed at –78 ºC. Et_3N (70 μL, 0.49 mmol) was added to a solution and the reaction mixture was stirred at 40 ºC for 45 min under nitrogen. Ether (100 mL) was added to the reaction mixture and the precipitate was filtered off to afford product **2** as a dark red solid. The residue was purified by flash chromatography eluting with 100 % EtOAc and 10 to 50 % MeOH/CH_2Cl_2 to afford product **2** as a dark red solid (43 mg, 45 %). R_f 0.5 (10 % MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (t, 1H, J = 13.2 Hz), 7.59 (d, 1H, J = 8.5 Hz), 7.51 (s, 1H), 7.43-7.38 (m, 2H), 7.30-7.27 (m, 2H), 7.16-7.13 (m, 4H), 7.04 (d, 1H, J = 13.0 Hz), 6.91 (d, 1H, J = 13.0 Hz), 5.97 (s, 2H), 4.15 (br, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 2.63 (br, 2H), 2.55 (s, 6H), 1.90 (br, 2H), 1.86-1.83 (m, 2H), 1.74 (s, 6H), 1.73 (s, 6H), 1.67-1.61 (m, 2H), 1.48 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 174.3, 173.6, 156.4, 155.2, 150.6, 142.9, 142.4, 141.7, 140.7, 140.6, 137.8, 132.7, 131.3, 129.7, 129.0, 125.7, 125.2, 125.1, 124.8, 124.5, 122.2, 121.0, 119.3, 114.0, 111.2, 110.9, 105.3, 105.1, 90.0, 89.8, 55.8, 49.2, 48.6, 44.7, 34.5, 32.3, 28.2, 28.1, 26.5, 26.0, 24.2, 14.6, 14.0; MS (MALDI) m/z calcd for $(M)^+$ $C_{52}H_{56}BF_2N_4O_3^+$ 833.44; found 833.29.



2

^{1}H NMR ($CDCl_3$)



^{13}C NMR ($CDCl_3$)

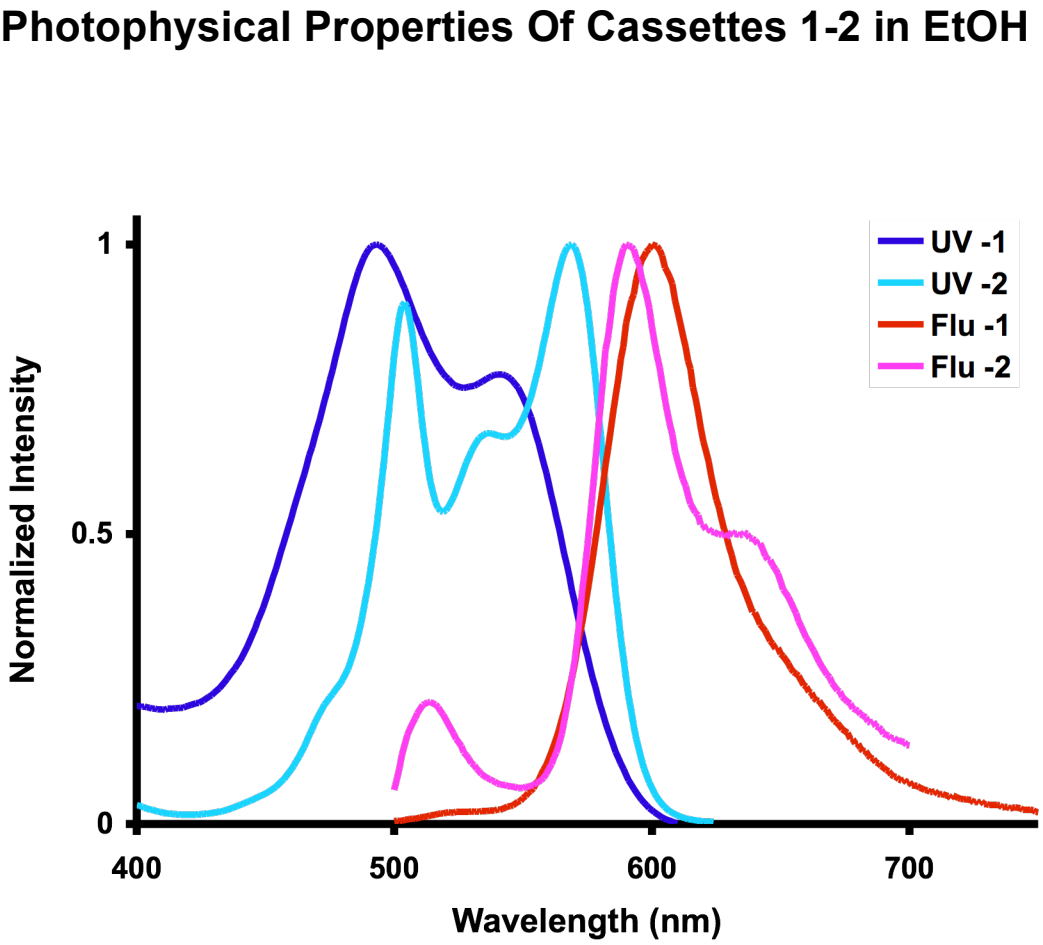


Figure S1. Normalized absorbance and fluorescence spectra of cassettes **1** and **2** in EtOH (at 10^{-6} and 10^{-7} M for absorbance and fluorescence measurements, respectively). All cassettes were excited at their corresponding donor absorption maxima.

Table S1. Energy transfer efficiency of cassettes **1** and **2** in EtOH.

dye	λ_{abs}(nm)	$\lambda_{em.}$(nm)	Φ_D[a]	Φ_A	ETE % (Φ_D/Φ_A)
1	498, 543	600	0.46+/-0.02	0.48+/-0.01[a]	96
2	504, 569	590	0.20+/-0.01	0.22+/-0.01[a]	90

Φ_D: quantum yield of cassette when excited at the donor absorption maxima. Φ_A: quantum yield of cassette when excited at the acceptor absorption maxima. Standards used for quantum yield measurement: [a]rhodamine 6G (Φ 0.92 in EtOH). Quantum yields were measured three times and averaged.

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In Vitro Cellular Imaging Studies

(a) Cell culture

Clone 9 normal rat liver cells (American Type Culture Collection) were cultured as subconfluent monolayers on 75 cm^2 culture flask with vent caps in Ham's medium supplemented with 10 % fetal bovine serum (FBS) in a humidified incubator at 37 ºC with 5 % CO_2. Cells grown to subconfluence were enzymatically dissociated from the surface with trypsin and plated 2-3 days prior to the experiments in Lab-Tek two well chambered coverglass slides (Nunc).

(b) Fluorescence microscopy

Uptake and subcellular localization of the cassettes **1** and **2** were studied on living Clone 9 normal rat liver cells using a Zeiss 510 META NLO Multiphoton Microscope System consisting of an Axiovert 200 MOT microscope. Throughout, digital images were captured with a 40x / 1.3 oil objective with the following filter sets:

- for cassettes **1** and **2** : Excitation 488 nm; Emission BP 500-530 for the donor part ; Emission BP 565-615 for the acceptor part

- for ER-Tracker™ Blue-White DPX : Excitation 740 nm; Emission BP 435 - 485

- for LysoTracker® Green DND-26 : Excitation 488 nm; Emission BP 500-530

*(b) Fluorescence microscopy for cassette **1**.*

Clone 9 cells were incubated for 30 min. at 37 ºC in serum free culture medium with 1 μM of cassette **1**. After the incubation period, the cells were washed with serum free culture medium several times before imaging.

To confirm the subcellular localization of cassette **1**, Clone 9 cells pre-treated with cassette **1** were co-incubated with 75 nM LysoTracker® Green DND-26 (1 mM stock solution, Invitrogen®) in serum free culture medium for 30 min at 37 °C. The cells were then washed with serum free culture medium before imaging (Figure S2).

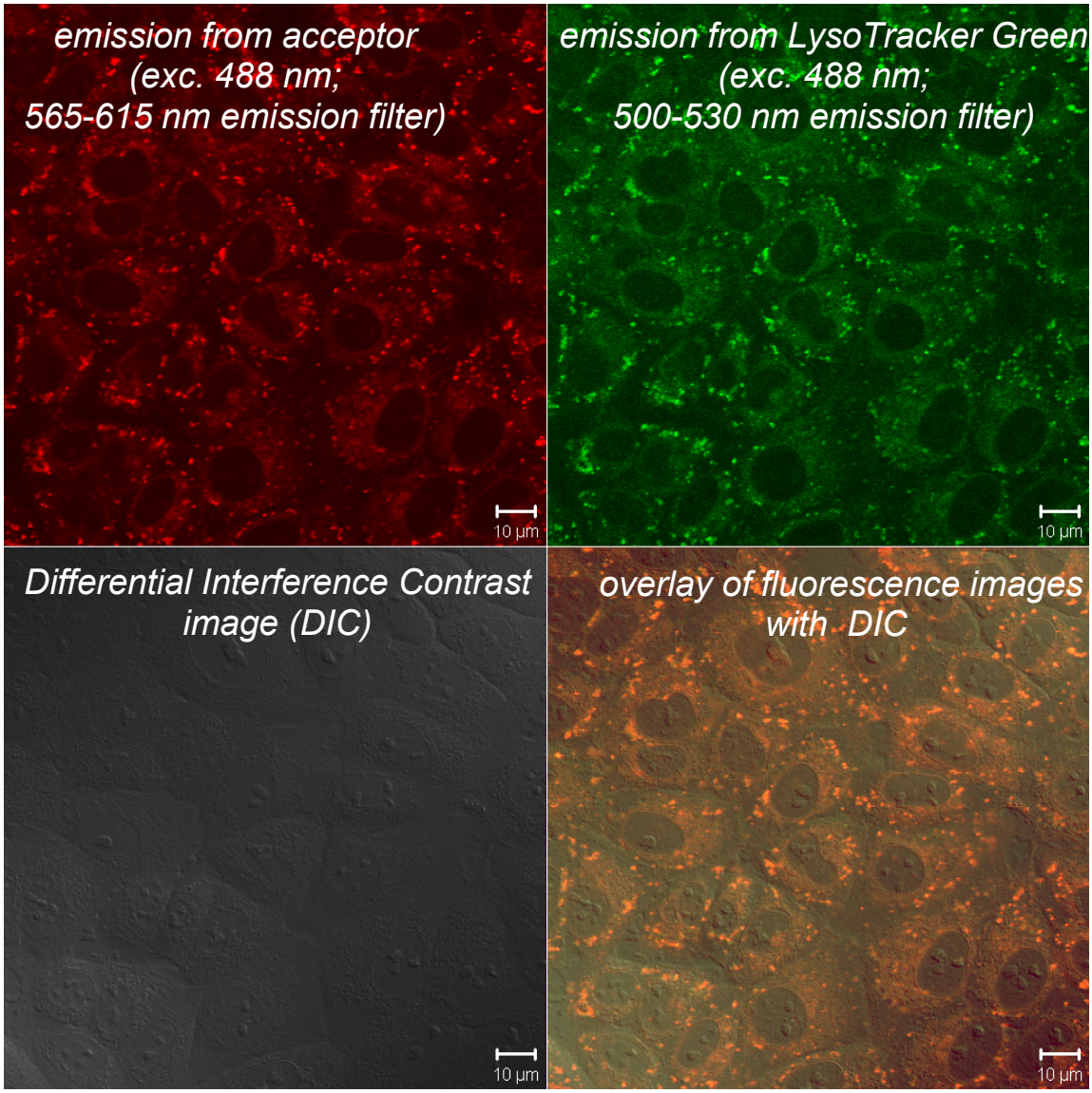

Figure S2. Cellular uptake of **1** and co-localization with LysoTracker® Green DND-26.

Thereafter, the same cells pre-treated with **1** and LysoTracker® Green DND-26 were co-incubated with 0.5 µM ER-Tracker™ Blue-White DPX (1 mM stock solution, Invitrogen®) in Hank's Balanced Salt Solution (HBSS) with Calcium and Magnesium for 30 min at 37 °C. After the incubation period, the cells were washed with serum free culture medium several times before imaging (Figure S3).

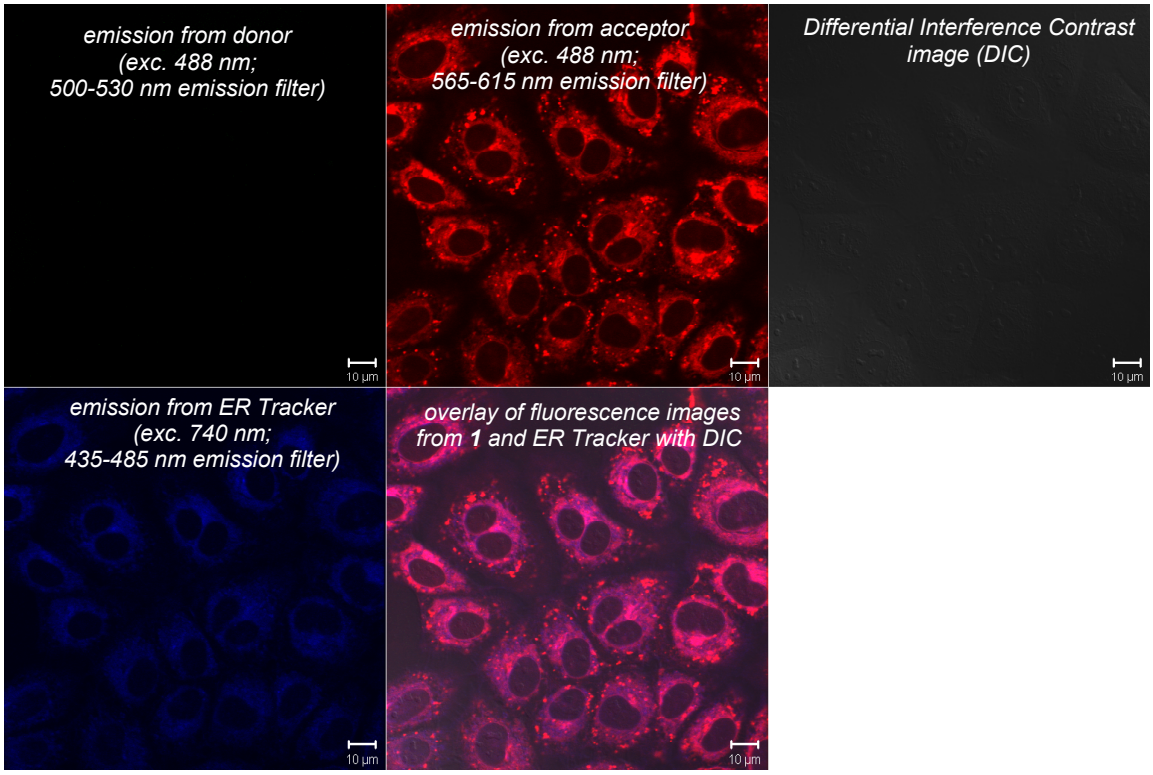

Figure S3. Cellular uptake of **1** and co-localization with ER-Tracker™ Blue-White DPX.

*(d) Fluorescence microscopy for cassette **2** (corresponds to Figure 2 in article).*

Clone 9 cells were incubated for 30 min. at 37 ºC in serum free culture medium with 1 μM of **2**. After the incubation period, the cells were washed with serum free culture medium several times before imaging (Figure S4).

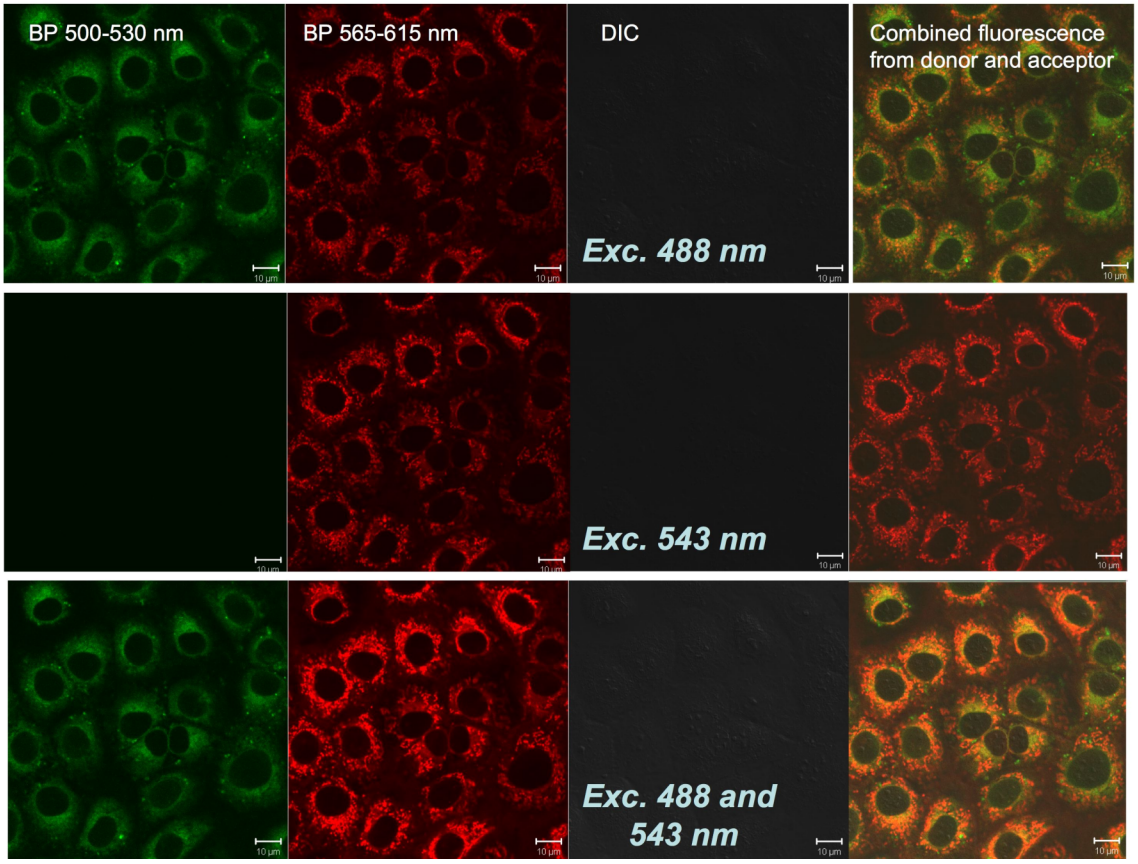

Figure S4. Cellular uptake of **2**. In the top panel, the cells were excited at 488 nm; **2** accumulated in the cytoplasm (green), lysosomes (green) and the mitochondria (red). In the middle panel, the cells were excited at 543 nm (acceptor part); the mitochondria are the only organelles labeled, suggesting that in the cytoplasm and the lysosomes, the fluorescence from the acceptor is quenched. The bottom panel is upon excitation at both 488 and 543 nm.

Clone 9 cells pre-treated with **2** were co-incubated with 0.5 μM ER-Tracker™ Blue-White DPX (1 mM stock solution, Invitrogen®) in PBS for 30 min at 37 °C (Figure S5).

The colocalization studies revealed that 37.41 ± 2.58 % of the *green* diffuse signal observed in the cytoplasm colocalized with the ER.

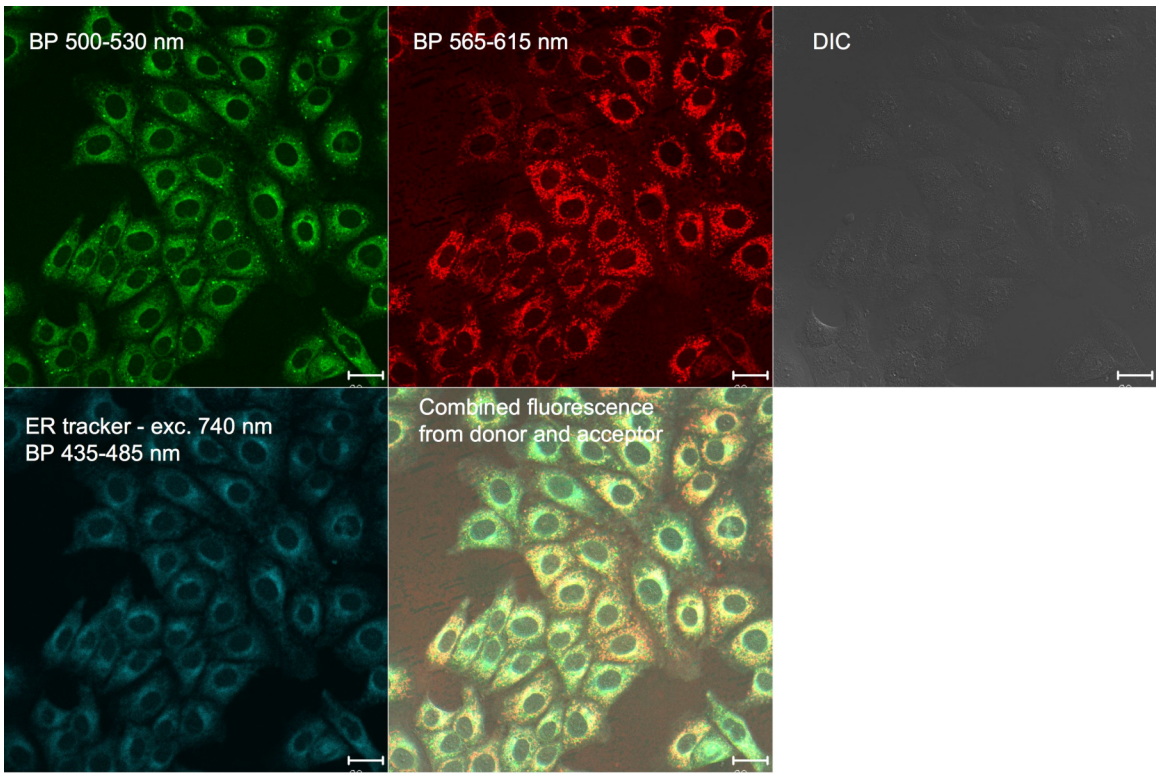

Figure S5. Cellular uptake of **2** and co-localization with ER-Tracker™ Blue-White DPX.

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